

Worker piping triggers hissing for coordinated colony defence in the dwarf honeybee *Apis florea*

Moushumi Sen Sarma¹, Stefan Fuchs^{2,*}, Christian Werber³ and Jürgen Tautz³

¹ Centre for Ecological Sciences, Indian Institute of Science, Bangalore, India

² Institut für Bienenkunde (Polytechnische Gesellschaft), Fachbereich Biology der J. W. Goethe-Universität, Frankfurt am Main, Germany

³ Lehrstuhl für Verhaltensphysiologie und Soziobiologie, Biozentrum der Universität Würzburg, Würzburg, Germany

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Summary

Defending a large social insect colony containing several thousands of workers requires the simultaneous action of many individuals. Ideally this action involves communication between the workers, enabling coordinated action and a fast response. The Asian dwarf honeybee, *Apis florea*, is a small honeybee with an open nesting habit and a comparatively small colony size, features that leave them particularly exposed to predators. We describe here a novel defence response of these bees in which the emission of an initial warning signal from one individual (“piping”) is followed 0.3 to 0.7 seconds later by a general response from a large number of bees (“hissing”). Piping is audible to the human ear, with a fundamental frequency of 384 ± 31 Hz and lasting for 0.82 ± 0.35 seconds. Hissing is a broad band, noisy signal, clearly audible to the human observer and produced by slight but visible movements of the bees’ wings. Hissing begins in individuals close to the piping bee, spreads rapidly to neighbours and results in an impressive coordinated crescendo occasionally involving the entire colony. Piping and hissing are accompanied by a marked decrease, or even cessation, of worker activities such as forager dancing and departures from the colony. We show that whereas hissing of the colony can be elicited without piping, the sequential and correlated piping and hissing response is specific to the presence of potential predators close to the colony. We suggest that the combined audio-visual effect of the hissing might deter small predators, while the cessation of flight activity could decrease the risk of predation by birds and insects which prey selectively on flying bees.

Key words: *Apis florea*, colony defence, worker piping, hissing, alarm communication

Introduction

Predation is a major hazard in the life of many animals. While solitary animals rely on behavioural mechanisms such as defending, escaping or hiding to diminish this risk, communication may play a major role in group-living animals. Birds may elicit warning calls signalling the need to hide, or to summon help for mobbing behaviour (Marler, 1955). In the Belding’s ground squirrel, animals warn communities to the benefit of their kin, even when it increases the risk to the caller (Sherman, 1977). Honeybee colonies consist of thousands of individuals and are particularly prone to predation as they accumu-

late considerable amounts of honey, pollen and bee brood. Defence against sudden attacks by large or organised predators requires the simultaneous action of many individuals. This would favour the evolution of a communication system in which the few individuals who detect the threat first, convey this information to as many individuals as possible and elicit immediate and coordinated defence responses from others. Such a system requires three features, namely signal specificity, fast transmission, and large amplification. As an example, the stinging response of honeybees uses an alarm pheromone to stimulate and focus attacks on the predator by other individuals (Koeniger et al., 1979).

*Corresponding author: Stefan Fuchs, Institut für Bienenkunde, J. W. Goethe-Universität, Frankfurt am Main, Karl-von-Frisch-Weg 2, 61440 Oberursel, Germany, phone: ++49-6171-21278; fax: ++49-6171-25769; e-mail: s.fuchs@em.uni-frankfurt.de

While most honeybee species build their colonies inside the cavities of trees or rocky crevices, which provide a considerable amount of protection, others construct colonies consisting of a freely accessible single comb. Colonies of the dwarf honey bee, *A. florea*, consist of a single vertically oriented comb suspended from a bush or tree branch. The upper broadened part of the comb incorporates the branch, and foraging bees depart and return from the flattened top of the comb which serves as dance floor for the recruitment of forager bees. The comb is completely enclosed in a living curtain of bees suspended from the overhanging edges of the broadened comb base and away from the surface of the comb, which cannot be seen.

Open-nesting species employ a number of behavioural strategies for colony protection which are less pronounced or entirely absent in the cavity-dwelling species. *A. florea* workers coat the branch which supports their colony with propolis to deter ant attacks (Seeley et al., 1982), and block temporary bridges across the bands of propolis formed by falling leaves or debris by holding these up with their heads (Sen Sarma et al., 2000). Upon direct mechanical disturbance of the colony *A. florea* produces a conspicuous hissing sound (Seeley et al., 1982). Hissing sounds are used by many animals as a defence strategy and are the most universal warning sounds. The broad band, mainly high frequency nature of these signals, suggests that they are designed primarily for vertebrate ears, and invertebrate producers of the sound might not be able to perceive it themselves.

In preliminary observations on *A. florea* it was noticed that hissing also occurred following the mere approach of a human subject and, significantly, that hissing in such cases was always preceded by a piping signal from one or a few individuals. Piping is a vibrational communication signal used extensively and in various contexts by honey bees, which is emitted by a single worker, usually while pressing her thorax against the wax comb, lifting the abdomen, and slightly spreading the wings. Piping individuals are frequently observed to walk around between consecutive pipings and the signals occur in the context of reproduction, foraging and swarming in the European honeybee *Apis mellifera* (Ohtani and Kamada, 1980; Pratt et al., 1996; Seeley and Tautz, 2001).

In this study we set out to investigate the piping and hissing phenomenon in terms of determining the identity of piping individuals, to quantify the temporal structure and vibration properties of the two signals and to explore the qualitative nature of the stimuli that lead to their release.

Materials and methods

Observations and experiments for this study were carried out at the Indian Institute of Science campus in

Bangalore, India. Colonies of the dwarf honeybee *Apis florea* (Fabr.) were collected from the neighbouring areas. Colonies were removed from the bush or tree by cutting through the supporting branch, transferring them to the campus, and then tying the ends of the branch to a support. Colonies were positioned approximately 1 to 1.5 m above the ground in a semi-shaded area and left undisturbed for 2 to 3 days. Experiments were carried out on 4 colonies (one colony was observed in its original site), in January 2001, 4 colonies in March and April 2001 and 1 colony in March 2002. Each colony contained brood cells, and had stored honey and pollen.

Video and audio techniques

All observations and experiments were recorded on digital video, unless stated otherwise. We used a Canon XL1 digital camera, either with a standard (50 mm) or with a tele-objective (Canon Video Lens 16× Zoom X1 5.5–88 mm IS 1:1.6–2.6 Ø with EF adaptor, and a Sony digital camera (DCR-TRV 7E). Sounds were recorded using a microphone (Sony ECM-959 A) fixed 4 to 5 cm above the colonies. Comb vibrations were recorded using Bruel and Kjaer accelerometers (type 4375 and type 4383, preamplifier 5935), fixed to the comb and left there over a few days to allow the bees to habituate to them. Airborne sounds or comb vibrations were recorded on the sound tracks of the video cameras.

Behavioural observations

Entire colonies were observed for a total of 3.5 hours and the occurrence of worker piping and hissing was stored on video. For closer examination of individual behaviour, returning workers were arbitrarily selected for focused video recordings. If the selected worker did not show piping behaviour within a minute of landing, we directed the video camera at another landing worker. Such focused animal sampling resulted in 6.5 hours of observations on video.

Releasing stimuli

Preliminary observations on the piping and hissing behaviour of *A. florea* suggested that a human subject approaching the hive was interpreted by the bees as a threat sufficient for the release of the warning. The effective stimuli could have been olfactory, visual, or a combination of both. For this study, the specific nature of the releaser was less important to us than the discovery of a stimulus that would reliably release the combined piping and hissing signals. Thus we mainly relied on a “human subject stimulus”, consisting of the subject walking slowly up to the colony and remaining motionless (as far

as possible) for various periods and at various distances from it, moving from behind a hide-out at different distances from the colony, or standing close to the colony but hidden from the view of the bees in the colony.

We additionally tried various stimuli, such as a “dummy” human subject (a black shirt), a 77×56 cm sheet of black paper (black optical stimulus) or a 8×30 cm strip of white tissue paper impregnated with butyric acid (odour stimulus). The dummy, optical and olfactory stimuli were drawn along a ropeway to a point 1 to 2 meters away from the colony, left there for a period of 5 or 10 minutes and then drawn slowly away to a distance of 8 meters from the colony.

Other stimuli we experimented with included mechanically tapping the branch on which the colony was supported, moving a black circular target (5 cm in diameter) about 1 meter from the colony, and directing three to four puffs of air at the bees in the colony through a plastic tube (inner diameter 2 mm). The behaviour of the bees was recorded in every case with digital video, microphones and accelerometers fixed to the comb as described above.

Data analysis

The sound track of the digital videotapes was transferred to a computer (16 bit sound card, sampling frequency 22050 Hz), saved as wav-files and analysed using Avisoft-SASLab Pro (Sound Analysis and Synthesis Laboratory, version 3.93) software. Piping series were analysed for the numbers of pipings within the series, the time interval between pipings, and the duration of pipings. The frequency and intensity structure of comb vibrations were analysed at a frequency resolution of 43 Hz (Hamming window). Comb vibrations of 50 hissing events were analysed similarly. Frequencies of piping and hissing behaviour over time were calculated using standard AV playback systems. The level of activity of the workers on the upper platform of the colony, such as arrivals, departures and dances, was measured from some of the video recordings. In 5 minute experiments, frequencies of piping and hissing were calculated omitting the first minute of the 5 minute intervals to avoid reactions extending into the following time interval. Statistical analyses were based on Sokal and Rohlf (1994). Wilcoxon’s matched pair signed rank test is abbreviated as WMPSR test.

Results

Behavioral observations

From recordings performed on 3 different colonies, 35 scenes were selected in which the behaviour of landing bees and that of surrounding workers was clearly visi-

ble. In 10 out of 23 observations landing workers were identified as foragers by their pollen loads. In two of the video scenes, behaviour after landing was analysed frame-by-frame, results of one are shown in Figure 1. After landing, workers emit a series of faint but audible piping sounds, adopting the typical worker piping body posture as described by Ohtani and Kamada (1980) and Pratt et al. (1996). During piping, they press the thorax against the comb and mostly change place between pipings. Surrounding workers frequently respond to piping by immediate hissing behaviour, which is propagated over the comb as a wave with a speed of 12.0 ± 6.4 cm/second ($N = 19$) as measured from the video recordings.

Temporal structure of piping and hissing and comb vibration properties

We analysed fourteen complete series of piping and hissing sequences elicited by single bees from separate recordings and found that piping and the following coordinated hiss was repeated from 3 to 15 times (median of pipings in a series = 7.5). Each of the coupled responses was interrupted by a pause of 2.84 ± 1.01 sec (min. = 0.48, max. = 5.13, $n = 98$) during which the pip-

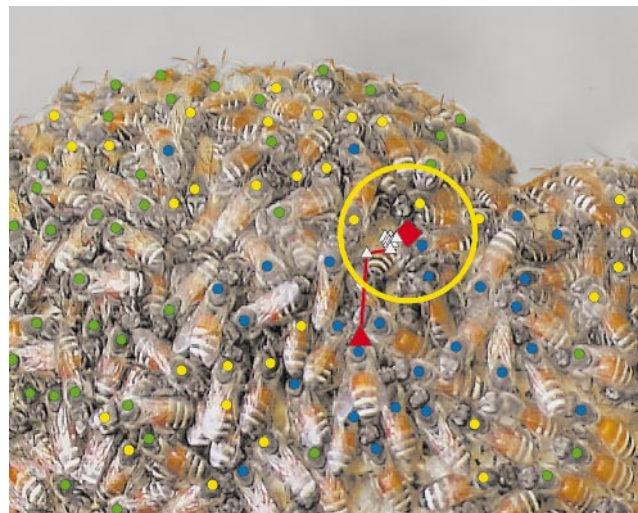


Fig. 1. A typical piping-hissing reaction reconstructed from a video sequence. A pollen forager lands at the upper slope of the comb (red triangle) and moves to the dance floor. 10.08 sec after landing it emits a series of 10 pipings at intervals of 3.67 ± 0.74 sec, each in a slightly different location (white triangles, red diamond). The still picture shows the video frame at the onset of the last piping (red diamond). 0.4 to 1.00 sec after onset of piping workers on the visible part of the comb showed a hissing response (median 0.68 sec, $N = 97$). Time of the worker reactions is indicated by colour coding ranging from early reactions (= blue, 1st terzil) to middle reactions (= yellow, 2nd terzil) to late reactions (green, 3rd terzil).

ing bee walked across the comb. The mean duration of each piping signal was 0.82 ± 0.35 sec (min. = 0.27, max. = 2.08, $n = 112$) and while this did not change within a series (Fig. 2, $r = 0.167$, $p > 0.05$), the intervals between pipings increased ($r = 0.267$, $p = 0.008$).

Of the 8125 piping events recorded in our experiments, 4783 were followed by hissing within the next second. However all of the recorded hissing events were preceded by the piping signal. The time interval between the onset of piping and onset of hissing was determined in 50 piping-hissing events, randomly selected from a comb vibration record, as 0.47 ± 0.11 (min 0.25 s, max 0.77 s), with the majority (64%) of intervals within a narrow time window of 0.3 to 0.5 s. The onset of hissing occurred mostly within the piping duration, and tight temporal coupling supports the causation of hissing by piping.

A sonogram of a piping-hissing series is shown in Figure 3. The piping signal sets up comb vibrations with a harmonic structure containing the fundamental frequency range of 310 Hz to 479 Hz (mean = 379 ± 35 Hz) and at least 5 subsequent harmonics (signals from 100 pipings, Fig. 4). The piping signal could also be picked up by a microphone, but the low signal to noise ratio of our recordings excluded an extensive analysis.

Hissing was accompanied by noisy broadband comb vibrations (90% of energy spectrum between 270–3600 Hz, $N = 25$) and was maintained for 1.0 to 1.8 seconds ($N = 50$). Hissing is not the result of superposition of nu-

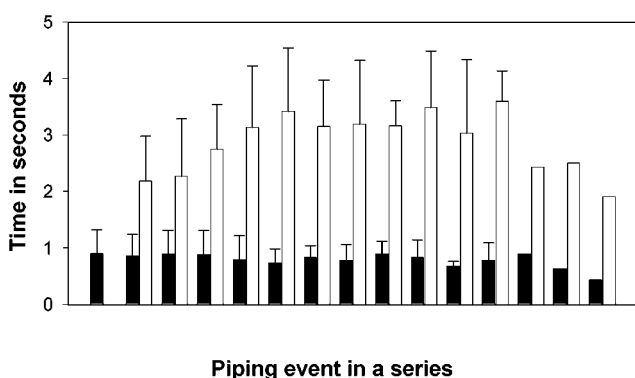


Fig. 2. The duration of the piping signal and the interval between consecutive signals in continuous series of up to 15 piping and hissing events. Filled columns indicate the duration of the piping signals and the open columns the time elapsed since the preceding piping. The figure is constructed from 14 recorded series containing 112 pipings, error bars show the time range. The number of series included are given above the respective pairs of columns, showing that up to three piping signals are common and long series of up to 15 consecutive signals are rare. While the duration of the piping signal does not change within series ($r = 0.17$, $P > 0.05$), the intervals between successive pipings increased slightly ($r = 0.267$, $P < 0.01$).

merous worker pipings, as shown by three instances where we were able to record isolated single bee hissings. Two of these were also broadband, low intensity sounds (224–882 Hz and 263–1985 Hz), the third one showed two peaks at 692.36 and 820 Hz.

Elicitation of coordinated piping and hissing

Release of coordinated piping and hissing by human subjects and other stimuli

Human subjects walked slowly and quietly to a spot 1–2 metres from the experimental colony, stood still for 10 minutes and then withdrew to a position 10 metres away. In these tests, a 10 minute period of exposure to the stimulus (human subject at the colony) was always paired with a 10 minute period of non-exposure (human subject 10 meters away). Such a test pair was repeated not more than 3 consecutive times with a given colony on a given day. As far as possible we standardised the approach of the human subjects in terms of how fast they approached the colony and withdrew from it.

The results of these tests is given in Figure 5 (top two graphs) which shows a typical series of three presentations and withdrawals of the human subject over a period of one hour. Piping and hissing activity increased significantly during the 10 minutes when the human subject was close to the colony, compared with the 10 minute periods when the human subject was 10 meters away from the colony (human subject close: pipings = 72.05 ± 34.11 , hissings = 60.41 ± 28.10 ; human subject distant: pipings = 16.82 ± 12.13 , hissings = 8.29 ± 8.02 ; $n = 17$, $p < 0.004$, WMPSR test). We concluded from these results that the presence of a human subject close to the colony was correlated in some way with the release of the coordinated response.

We extended the human subject tests by using the dummy (shirt), black optical (cardboard) and olfactory (butylic acid) stimulus in an attempt to abstract from the complexities of the human subject. All these alternative stimuli were drawn up to the colony on a ropeway and when near the colony remained completely still. Exposure and non-exposure of the colony to the stimuli was paired as before with 10 minutes of exposure (stimuli close to the colony) alternated with 10 minutes of non-exposure (stimuli 8 meters away from the colony).

Piping and hissing increased significantly during the 10 minutes when the dummy was close compared to the 10 minutes when it was 8 meters away (dummy close: pipings = 64.8 ± 7.33 , hissings = 32.6 ± 5.42 ; dummy distant: pipings = 43.8 ± 10.51 , hissings = 13.00 ± 3.79 ; $n = 5$, $p < 0.03$, WMPSR test). However, very low levels of piping and hissing were recorded during the 10 minutes when the black optical stimulus was close compared to the 10 minutes when the target was 8 meters away (optical stimulus close: pipings = 22.00 ± 14.96 , hissings =

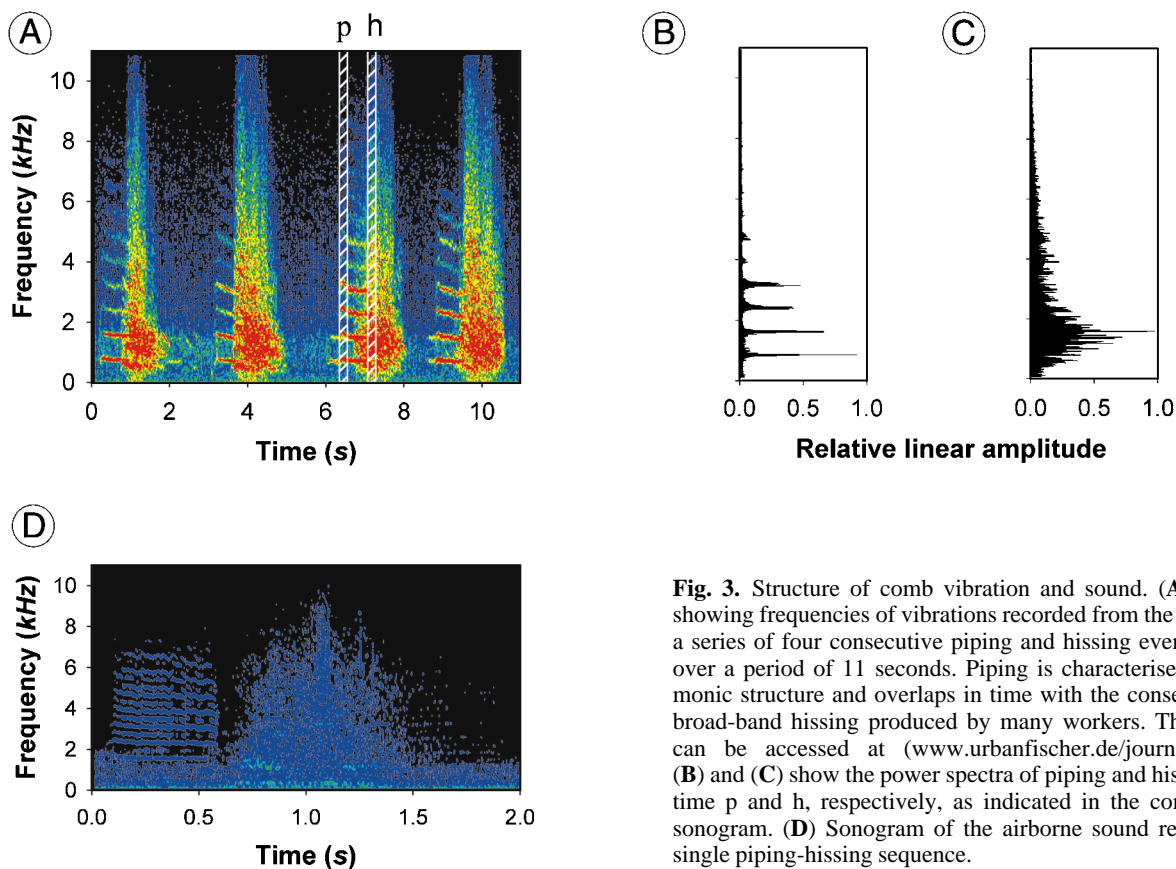


Fig. 3. Structure of comb vibration and sound. (A) Sonogram showing frequencies of vibrations recorded from the comb during a series of four consecutive piping and hissing events occurring over a period of 11 seconds. Piping is characterised by its harmonic structure and overlaps in time with the consecutive noisy broad-band hissing produced by many workers. The sound file can be accessed at (www.urbanfischer.de/journals/zoology). (B) and (C) show the power spectra of piping and hissing taken at time p and h, respectively, as indicated in the comb vibration sonogram. (D) Sonogram of the airborne sound recording of a single piping-hissing sequence.

0.66 ± 0.94 ; optical stimulus distant: pipings = 10.00 ± 1.63 , hissings = 1.00 ± 0.82 , $n = 3$, ns). Negligible levels of piping and hissing were recorded when the olfactory stimulus was offered to the colony (odour close: pipings = 4 ± 3.78 , hissings = 1.66 ± 2.62 , odour distant: pipings = 5.66 ± 3.94 , hissings = 0.83 ± 1.86 , $n = 6$, ns). We suspected that responses during periods when the stimulus was absent could have been caused by general disturbances due to setting up the experimental devices, and recorded a colony left undisturbed over 2 days. Background activity was considerably lower and only 4 piping-hissing bouts occurred over 50 min (0.82 piping/min, 0.56 hissing/min).

We concluded from this series of tests that a relatively large, but not necessarily moving object in the vicinity of the colony can, but not necessarily does, capture the essential features needed to release the coordinated piping and hissing response in the colony. Odour, in form of the pure butyric acid from the olfactory stimulus, is apparently not on its own a releaser of the warning signal.

Distance of releaser

Piping and hissing increases when human subjects stand close to the colony, compared to when they stand at a distance from it, suggesting that the closer the subject

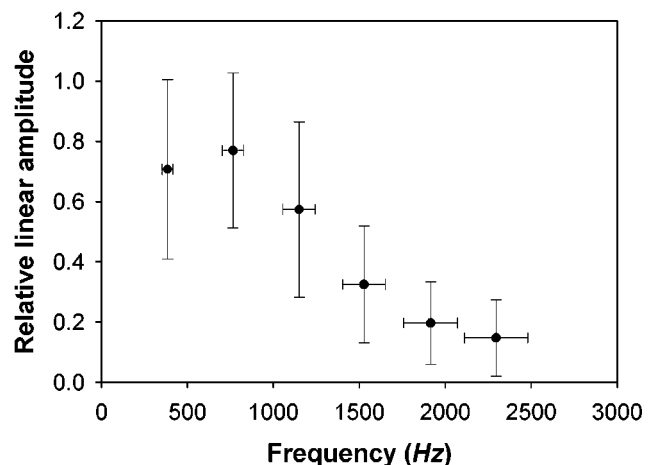


Fig. 4. Mean frequency and relative linear intensity of the fundamental frequency (leftmost score) and subsequent harmonics analysed from 100 separate piping signals. Frequency means of the harmonics differed by less than 0.53% from integer multiples of the fundamental, and increase in standard deviations was in accord with that expected from the variation in the fundamental. Means and standard deviations of relative linear intensities were calculated relative to the highest value in each piping set to 1. The relative linear intensity of the fundamental did not differ from that of the first two harmonics, while that of the following three harmonics was lower ($P < 0.0005$).

stands to the hive, the greater the perceived threat. We tested the effect of distance of the stimulus on the response by first selecting stations at distances of 1, 3, 5, 7, 9 and 11 meters from the hive. A human subject stood still at one of these stations for 5 minutes and then retired behind a screen for another 5 minutes so that it was no longer visible, and then walked to the next station. Complete series over all stations were repeated six times, starting either at the smallest distance or the largest distance. We found that the reaction of the bees to

human subjects diminishes rapidly as their distance from the colony increases but that even at 9 meters, significantly more coordinated piping and hissing responses occurred during the 5 minutes that the human subject was visible in comparison to when they were hidden behind the screen (Table 1, $n = 6$, $p < 0.03$, WMPSR test).

Role of foragers: Is exposure of foragers to the stimulus sufficient to elicit coordinated piping and hissing?

Coordinated piping-hissing can be effected by returning foragers, as evidenced from behavioural records and analyses. In the tests above, the presence of the releasing stimulus, whether human subject or dummy, was clearly visible to the bees on the colony and also to those departing from and returning to the colony. Thus it was not clear whether the behaviour required that the stimulus be perceived from the bees on the comb, or whether it was sufficient that foragers were exposed to the stimulus during their return. To test this, a colony was surrounded on all sides by plywood sheets, reaching from the ground to a height exceeding the top of the colony by about 50 cm. Flying bees returning to the colony could approach and land on it through the opening at the top of the walled-in area. A human subject was stationed at 1m from the colony and located so as to be invisible to the individuals in the colony, but visible to flying bees as they approached to land on the colony. As before, the bees were exposed to the human subject close to the colony for a period of time and their response compared with periods when the human subject was stationed at 10 meters from the colony. Exposure for 5 minutes was paired with non-exposure for 5 minutes and such a pair was repeated 12 times.

The result of this series of tests showed that piping and hissing increased significantly during the 5 minutes when a human subject was standing close to the colony, compared to the 5 minutes when the observer was distant from it, although the human subject was not visible at either station to the bees on the colony (human subject close: pipings = 54.25 ± 19.56 , hissing =

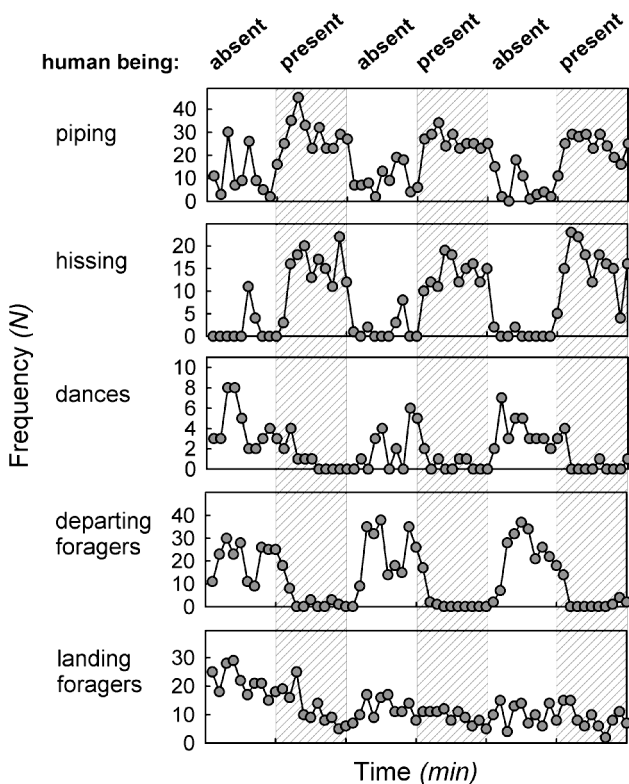


Fig. 5. Piping and hissing behaviour and the location of a human subject relative to an *A. florea* colony is shown in the upper two graphs. The shaded columns represent consecutive 10 minute periods of a continuous 60 minute recording session. Non-shaded columns indicate the time during which the human subject was far from the colony at a distance of 10 meters, shaded columns the time during which the human subjects were close at 1 to 2 meters. In all cases the human subjects were standing still. The frequency of the piping and hissing in each minute of the continuous recording are shown by the points in the columns. There are significantly more piping and hissing events when human subjects stand close to the colony than when they were located 10 meters away from it. The relationship between colony activity (dancing, departures, arrivals) and the location of a human subject relative to an *A. florea* colony is shown in the lower three graphs. Dancing activity is depressed during the time the human subject is close to the colony and few, if any, bees depart from the hive in comparison to the periods when the human subject is 10 meter away from the colony. The landing frequency of foragers is less influenced but tends to decrease over the first 10 minutes of the 60 minute observation period.

Table 1. Effect of distance of a human subject from a colony of *Apis florea* on the frequency of piping and hissing behaviour within 4 min observations (6 repetitions per distance).

| Distance (metres) | Human being present (4 minutes) | | Human being absent (4 minutes) | |
|-------------------|---------------------------------|---------------|--------------------------------|-------------|
| | Piping | Hissing | Piping | Hissing |
| 1 | 55.16 ± 15.06 | 42.16 ± 11.10 | 9.83 ± 6.43 | 2.16 ± 4.41 |
| 3 | 29.50 ± 14.41 | 16.16 ± 16.81 | 4 ± 3.31 | 0.83 ± 1.21 |
| 5 | 27.50 ± 10.66 | 16.83 ± 9.65 | 4.66 ± 5.73 | 0.16 ± 0.37 |
| 7 | 11.83 ± 6.31 | 7.5 ± 7.01 | 4.66 ± 4.46 | 1.33 ± 2.21 |
| 9 | 9.83 ± 6.86 | 4.5 ± 4.03 | 4.83 ± 8.25 | 0.83 ± 1.86 |
| 11 | 8.83 ± 7.58 | 4.33 ± 5.00 | 4.83 ± 7.53 | 0.5 ± 1.11 |

40.25 ± 10.60; human subject distant: pipings = 8.33 ± 11.93, hissings = 2.41 ± 4.27, n = 12, p < 0.03, WMPSR test). Repeating the tests with the dummy instead of the human subject produced essentially the same result: piping and hissing both increased significantly during the 5 minute periods with the dummy close to the colony, compared to 5 minute periods when it was distant (dummy close: pipings = 64.8 ± 7.3, hissings = 32.6 ± 5.4; dummy distant: pipings = 43.8 ± 10.5, hissings = 13.0 ± 3.8; n = 6, P < 0.05, WMPSR test).

We conclude from these results that the coordinated piping hissing is initiated by foragers perceiving the releasing stimulus during their return to the colony. The stimulus does not need to be perceived during piping or hissing.

Role of foragers: Is the initiation of coordinated piping and hissing restricted to returning foragers?

To test whether bees returning to the colony are the only bees responsible for the initiation of the coordinated warning signal, we enclosed a colony within a 150 × 150 × 200 cm net for 5 minutes which prevented returning bees from alighting on the colony. After 5 minutes the net was lifted off the colony to allow returning bees access to it for a period of 5 minutes. During this time, two observers stood still, 1–3 metres from the colony. Periods with and without the net enclosure were paired and repeated 6 times, resulting in a total of 12 repetitions over 2 hours distributed over two days on one colony. The test was then repeated but with the net being raised and lowered with a system that could be operated remotely by the observers who were completely hidden from the view of both the returning bees and those on the colony, and located 10 meters away from the colony.

We found that when forager bees were excluded from the colony by a net for 5 minutes, piping and hissing (counted over the last 4 minutes) was almost completely absent although the experimenters stood at a distance of 1m from the colony but increased significantly as soon as foragers were admitted (colony enclosed: pipings = 3.9 ± 3.5, hissings = 0.2 ± 0.4; colony open: pipings = 69.1 ± 17.8, hissings = 50.3 ± 0.2, n = 12, p < 0.05, WMPSR test). In the control experiment, where the cage was closed and opened in the absence of a human subject, we found no significant difference between the number of piping and hissing sequences in an enclosed colony and in an open colony (colony enclosed: pipings: 6.75 ± 5.63, hissings = 0.66 ± 1.69; colony open: pipings = 5.25 ± 2.97, hissings = 0.58 ± 1.65; n = 12, p > 0.05, WMPSR test). The control experiment shows that piping and hissing was not released by lifting the cage.

As reported previously for *A. florea* (Seeley et al., 1982), mechanical disturbance of the colony will result in a hissing response from the colony. We thus also

tested whether coordinated piping and hissing would result from direct colony disturbances. Tests were carried out in the mornings before flight activity had set in on four colonies over a period of 10 days. We found that one or two gentle taps on the branch supporting the colony elicited hissing that spread over the entire colony in 10 out of 10 trials, but no piping behaviour was recorded. A black cardboard disc (diameter 5 cm) fixed to a 50 cm wooden handle was waved up and down 5 times at a distance of approximately 1 m from the colonies. This moving optical stimulation did not release piping or hissing behaviour but bees did react by sidewise body shaking in all 10 trials. Optical stimulation was followed by blowing puffs of air through a plastic tube at some of the bees on the colony. Air puffs induced a local hissing response in 10 out of the 10 trials, largely restricted to the stimulated individuals, but on two occasions the hissing spread outwards and elicited hissing from bees that had not themselves received the stimulus. Again no piping was recorded.

We concluded that coordinated piping and hissing depends on returning foragers and is not initiated by other bees in the colony.

Colony activity and presence of a human subject near the colony

Piping and the coordinated warning signal are not the only responses of the bees to the presence of a human subject near the colony. Recordings of dancing and flight activity (departures and landings) showed that these activities also decreased significantly when a human subject stood close to the colony. The results from a series of 10 minute paired exposures to human subjects at stations close to and 10 meters distant from the colony (Fig. 5, bottom three graphs), show that dancing and departure activity decreased significantly in the 10 minutes when a human being was close to the colony compared to the 10 minutes when the human being was 10 meters away (human subject close: dances = 5.20 ± 10.17, departures = 31.47 ± 35.60; human subject distant: dances = 15.05 ± 15.14, departures = 94.58 ± 71.2; n = 17, p < 0.004, WMPSR test). Using a shielded colony and dummy stimulation, dancing and departures also decreased (shirt present: dances = 3.8 ± 2.85, departures = 25 ± 17.17, shirt absent: dances 5.4 ± 1.45, departures = 42 ± 5.25; N = 5) though due to only few repetitions this decrease was not significant.

Discussion

Colonies of the Asian dwarf honeybee *Apis florea* react to the presence of a stationary human subject or a dummy close to their colonies with a hissing signal.

This hissing behaviour is released by an initial piping signal from one to several individuals and involves the majority of the bees in the colony.

That hissing in *A. florea* is elicited by piping and not independently by some other cue (olfactory or visual) is supported by a number of our observations. First, there is a close temporal association (0.29 to 0.71 s) between piping and the subsequent hissing. Second, all hissings elicited by closeness of humans to the colony were preceded by piping ($n = 4783$). Third, hissing can be elicited by mechanical stimulation such as tapping the colony support, but not by the mere closeness of humans, as evidenced by the absence of hissing in the colony stimulation experiments and in colonies covered by netting.

Observations and video recordings showed that piping was performed by returning foragers. Excluding foragers by enclosing the colonies in a net cage, or colony stimulation in times without flight activity, confirmed that other workers are extremely unlikely to initiate the coordinated piping and hissing sequence. In addition, it is sufficient that returning foragers perceive the human or other stimulus on their return to the colony, even if this is invisible from the colony. Thus returning foragers are believed to carry the information to the colony, and we propose that they convey this information through piping.

Preliminary experiments with dummies did not shed much light on the exact nature of the configuration of stimuli eliciting the behavior but strongly suggests a pronounced optical component. However, while a black shirt elicited reactions a black sheet of paper did not, indicating some specificity to the stimulus. Odour does not seem to be a key feature, as indicated by the absence of reactions to humans in experiments with shielded or caged colonies and with butyric acid odour baits, a component contributing to the odoriferous nature of human sweat.

Piping *A. florea* workers adopt a posture similar to *A. mellifera* while piping and also similar to the *A. mellifera* foragers described by Pratt et al. (1996), walk in a circuitous manner across the comb in between piping and the piping signal produced by the two species has a similar mean duration (0.8 s and 1.0 s). However, the mean fundamental frequency of piping signals emitted by *A. florea* (384 Hz) was closer to that emitted by *A. mellifera* workers, 350 and 380 Hz, in the context of laying and foraging respectively (Ohtani and Kamada, 1980; Pratt et al., 1996), but is much lower than the fundamental frequency of pipings (500 to 700 Hz) emitted by guard bees of *A. mellifera* (Ohtani and Kamada, 1980). The structure of the comb vibration caused by piping by *A. florea* individuals is also similar to that found in *A. mellifera* by Ohtani and Kamada (1980), and Pratt et al. (1996), consisting of a fundamental frequency and at least 5 distinct harmonics. In many cases, the intensity of the fundamen-

tal frequency was lower than that of the subsequent harmonic, probably due to the complex transmission properties of the comb (Sandeman et al., 1996). The intensity of the piping signal decreased with the distance between the piping bee and the transducer we used to measure it, and recorded amplitudes were near zero at distances greater than 10 to 15 cm from the source. Whereas the piping signal in *A. florea* has now been shown to be most likely part of a warning signal, this possibility has not yet been considered for *A. mellifera*. We cannot, however, exclude the possibility that some of the recorded piping signals might have served other purposes.

Simultaneous hissing of many bees in a colony is a signal that is employed by a number of bee species. *A. cerana* colonies hiss in response to mechanical disturbances, substrate vibrations and airborne sounds (Fuchs and Koeniger, 1974). Air movement or substrate vibrations elicit hissing in *A. mellifera* (Spangler, 1986). Hissing signals could have at least two functions. One of these is to warn the bees themselves of a potential threat. Hissing is often followed by the decrease or temporary suppression of colony activity which in bees such as *A. florea* with exposed colonies may alter the appearance of the colony, making it less conspicuous. In *A. mellifera*, there are reports of complete cessation of activity (termed freezing) occurring when substrate vibrations at frequencies between 500 and 1000 Hz were applied to the hives (Little 1962) and of complete suppression of flight activity (Spangler, 1969) by continuous vibration of the comb at 600 Hz. Cessation of forager departure from hives would lead to an increase in the number of workers who could defend the colony, if necessary. Cessation of flight activity and hence a reduction in the number of flying bees would be adaptive if birds were hovering nearby to snatch single flying bees, rather than threatening the entire colony. Up to 30% of the crop content of bee-eaters like *Merops orientalis* and *Merops superciliosus* were found to be composed of *A. florea* bees (Fry, 1984).

The coordinated hissing of a bee colony probably functions secondly as a warning to deter potential predators. Such a function has been suggested for hissing bumblebees (Kirchner and Röschard 1999) and *A. cerana* and *A. dorsata* (Fuchs and Koeniger, 1974; Seeley et al., 1982).

We propose here that *A. florea* foragers upon returning to their nest are able to recognise particular objects in the vicinity of the nest that could represent a threat to the colony. On alighting these returning bees initiate, through piping signals applied to and transmitted through the comb, a hissing response from nearby workers which initiates a coordinated hissing response by a significant number of the colony members. Hissing, in turn, is assumed to serve as a deterrent, and by quieting the colony's activities, is thought to protect the

colony. The frequency of coordinated piping and hissing may be an indication of the intensity of the threat, reflected in the number of returning bees that perceived it and the intensity of their piping.

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